Nifekalant Hydrochloride Administration During Cardiopulmonary Resuscitation Improves the Transmural Dispersion of Myocardial Repolarization
— Experimental Study in a Canine Model of Cardiopulmonary Arrest —

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Background
Because nifekalant hydrochloride (NIF) displayed a superior defibrillating effect on ventricular tachycardia/fibrillation (VT/VF) in cardiopulmonary arrest (CPA) patients, despite some QT prolongation, its effect on transmural dispersion of repolarization (TDR) in the left ventricle (LV) in an animal model of CPA was investigated.

Methods and Results
Eight beagle dogs were created with a myocardial infarction under anesthesia, and then VT/VF induction by continuous stimulation and cardiopulmonary resuscitation (CPR) were repeated. NIF (0.3 mg/kg) was administered under acidic conditions (pH 7.26). The QTc interval measured by Y-lead ECG showed no significant prolongation before and after NIF. The activation recovery interval (ARI) measured by 64-lead LV surface mapping showed minimum ARI prolongation (40%) by NIF without maximum ARI prolongation, and as a result the ARI dispersion decreased by 67%. The repolarization time (RPT) with the plunge electrode showed 13–19% prolongation in the subendocardium and subepicardium with CPR, but NIF prolonged the RPT in the middle layer alone (17%), and as a result Plunge-TDR decreased by 82% (n=8, p<0.05).

Conclusions
Administration of NIF during CPR decreased the TDR by RPT prolongation selectively in the middle layer. Because the subendocardial and subepicardial RPTs after CPR were already prolonged before NIF administration, it may have been the reason why the QT-prolonging effect of NIF was not reflected in the body surface ECG. (Circ J 2006; 70: 1200–1207)

Key Words: Activation recovery interval; Cardiopulmonary resuscitation; Nifekalant hydrochloride; QT interval; Transmural dispersion of repolarization
Effect of NIF During CPR on the TDR of Ventricle

very effective, even under conditions of severe acidosis such as occur in OHCPA. However, the effect of the QT-prolonging action of NIF was significantly suppressed in OHCPA compared with IHCPA, and the VT/VF suppressive effect was difficult to explain by a QT-prolonging action. To explain the mechanism by which VT/VF was stopped despite the poor QT-prolonging effect, we hypothesized that NIF administration during cardiopulmonary resuscitation (CPR) improved the epicardial surface and transmural dispersion of myocardial repolarization. We therefore created a model of cardiopulmonary arrest (CPA) in dogs and used it to systematically investigate the repolarization time (RPT) in the LV.

Methods

General Procedure

Eight adult beagle dogs (body weight: 8–10kg) were used. Endotracheal intubation was performed after induction anesthesia with thiamylal (15 mg/kg iv), and artificial ventilation was started. The anesthesia was maintained with α-chloralose (50 mg/kg, iv + Div). A catheter was inserted through the left femoral artery and used to monitor blood pressure and measure arterial blood gas. With the dog in the right lateral position, thoracotomy was performed in the 5th intercostal space, and the heart was lifted superiorly by a pericardial tent. The connective tissue around the proximal branch of the left anterior descending artery (LAD) was dissected, the trunk of the artery was ligated with a silicone band to create AMI (Fig 1A). A bipolar electrode was attached to the left atrium and to the LV, high-frequency stimulation (150/ms) was applied to the LV, and induction of VT/VF and CPR were repeated. Before and after CPR, body surface Y-lead ECG and 64-lead left ventricular surface potential mapping (64 Map) were performed. In 4 of the 8 animals a plunge electrode was used to record the potentials in the epicardial layer, middle layer, and endocardial layer of the LV wall (Fig 1B). The RR interval and QT interval were measured by the Y-lead ECG (FDX-6521-A, Fukuda Denshi, Tokyo, Japan). The recordings were made with monopolar leads.
plunge electrode was inserted into the epicardium of the LV wall directly below the point where D1 branched off the LAD (Fig 1A, B). The plunge electrode was a 1-mm long silver wire, and wire arranged every 3 mm on a 50-gauge Teflon-processed steel wire was used. The recordings were made with monopolar leads.

Two SCXI-10032-channel multiplexer modules (National Instruments) were used as the multiplexer and amp units for potential recording, and an output range amplification rate of 1,000-fold (60dB) was used. A PCI-MIO-16E-4 A/D card (National Instruments) was used. Resolution was 12 bits, and sampling frequency was 250 kS/s. The analog filter settings were 0.05 Hz (passive CR, 1st-order) to 500 Hz (active Butterworth, 2nd-order). The data were analyzed on an IBM-PC AT compatible PC with Lab VIEW (National Instruments), Excel2000 (Microsoft), and HMS-100 (Unique Medical) software.

**Experimental Protocol**

After creation of the AMI, VT/VF was induced by electrical stimulation, and cardiac massage, epinephrine administration, and direct current stimulation were repeated for CPR. Arterial blood gas analysis was performed on blood collected from the left femoral artery catheter every 10 min, and NIF (0.3 mg/kg, iv) was injected when metabolic acidosis (pH <7.3) had been achieved (Fig 1C). A dose-escalation was collected from the left femoral artery catheter every 10 min, and was dose-dependent in a canine AMI model.7

### Table 1 The pH and Lactic Acid of Arterial Blood as the Parameters of Acidosis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ligation 30 min</th>
<th>Ligation 60 min</th>
<th>Ligation 90 min</th>
<th>Ligation 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum pH</strong></td>
<td>7.38±0.05</td>
<td>7.39±0.05</td>
<td>7.29±0.10*</td>
<td>7.16±0.10*</td>
<td>7.14±0.06*</td>
</tr>
<tr>
<td><strong>Lactate (mg/dl)</strong></td>
<td>0.9±1.8</td>
<td>10.1±2.9</td>
<td>46.1±29.2*</td>
<td>65.6±23.3*</td>
<td>79.1±22.4*</td>
</tr>
</tbody>
</table>

Values are mean±SD in dogs (n=8). Data were obtained before and during cardiopulmonary resuscitation and after successful defibrillation. *Significant value <0.05 vs control.

### Table 2 Effect of NIF on RR Interval and QT Interval Evaluated by Y-Lead ECG

<table>
<thead>
<tr>
<th></th>
<th>Plunge</th>
<th>Ligation 1 h</th>
<th>Ligation 10 min</th>
<th>Post CPR</th>
<th>After NIF iv</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RR (ms)</strong></td>
<td>401±43.8</td>
<td>413±41.9</td>
<td>414±43.4</td>
<td>400±37.5</td>
<td>422±55.7</td>
</tr>
<tr>
<td><strong>QT (ms)</strong></td>
<td>208±18.9</td>
<td>217±21.4</td>
<td>214±18.7</td>
<td>209±19.9</td>
<td>202±29.0</td>
</tr>
<tr>
<td><strong>QTc (B)</strong></td>
<td>327±16.0</td>
<td>338±16.8</td>
<td>332±13.9</td>
<td>330±15.9</td>
<td>337±22.4</td>
</tr>
<tr>
<td><strong>QTc (F)</strong></td>
<td>281±17.6</td>
<td>291±19.2</td>
<td>287±16.0</td>
<td>283±18.0</td>
<td>292±25.7</td>
</tr>
<tr>
<td><strong>QTc (VDW)</strong></td>
<td>260±15.9</td>
<td>268±17.8</td>
<td>265±15.2</td>
<td>261±16.7</td>
<td>270±24.2</td>
</tr>
</tbody>
</table>

Values are mean±SD in dogs (n=4). Data were obtained before and during coronary ligation, and nifekalant hydrochoride (NIF) infusion. CPR, cardiopulmonary resuscitation; QTc (B), QT/(HR) 1/2 by Bazett; QTc (F), QT/(HR) 1/3 by Fridericia; QTc (VDW), QT-0.087 (RR-1000) by Van de Water.

Experimental Protocol

After creation of the AMI, VT/VF was induced by electrical stimulation, and cardiac massage, epinephrine administration, and direct current stimulation were repeated for CPR. Arterial blood gas analysis was performed on blood collected from the left femoral artery catheter every 10 min, and NIF (0.3 mg/kg, iv) was injected when metabolic acidosis (pH <7.3) had been achieved (Fig 1C). A dose-escalation study reported that the QT-prolonging effect of NIF (0.1–1.0 mg/kg) was manifested at the lowest dose, 0.1 mg/kg, and was dose-dependent in a canine AMI model.

**Body Surface Y-Lead ECG**

The averages values of the RR and QT intervals measured in 6 consecutive heartbeats by body surface Y-lead ECG were recorded and the QT interval was corrected by using the formulae proposed by Bazett (QTc(B)), Fridericia (QTc(F)), and Van de Water (QTc(VDW)).

**Sixty-Four-Lead LV Surface Potential Mapping**

Activation time (AT), recovery interval (RI), activation–RI (ARI), dispersion of activation RI (ARID), and TDR estimated by 64 Map (Map-TDR) in the LV were evaluated by 64 Map. AT is defined as the electronic differentiation from the beginning of the QRS to the minimum dv/dt of the QRS wave, and the minimum AT (min-AT) value in the total measurement region was assumed to be 0. Conduction time, the difference between the maximum AT (max-AT) and min-AT, was used as an index of excitation–conduction time, and its reciprocal was defined as conduction velocity (CV). ARI is the difference obtained by subtracting AT from the position of maximum differential value of the T wave, and it was used as an index of the local refractory period. RT extends from the start of the QRS wave to the maximum differential of the T wave (ie, sum of AT and ARI) and it was used as an index of the time from the depolarization to repolarization. All of the measurements were made in 3 consecutive heartbeats during sinus rhythm, and the mean values were calculated. ARID was calculated as the difference between the maximal ARI (max-ARI) and the minimum ARI (min-ARI) in the 64 measurement regions, and it was used as an index of the 2-dimensional dispersion on the heart’s surface. In addition, the differences between the peak of T wave (Tpeak) and the end of T wave (Tend) at 4 fixed points were calculated, and the mean values were extracted and used as an indirect evaluation of the TDR.

**Electric Potentials With the Plunge Electrode**

The recordings using the plunge electrode were made with Y-lead ECG and 64 Map at 6 points in time: immediately after insertion of the plunge electrode, 1h after insertion of the plunge electrode, when the injury current had disappeared, immediately after coronary artery ligation, 10 min after coronary artery ligation, after CPR, and 10 min after intravenous injection of NIF (Fig 1C). In view of the occurrence of the injury current associated with plunge insertion, we used the values 1 h later as the baseline. The potentials obtained with the plunge electrode were used to measure the RPT in the subepicardium, middle layer and subendocardium, and the TDR estimated by plunge electrode (Plunge-TDR) was calculated. RPT was defined as the difference obtained by subtracting AT from Tend, and Plunge-TDR was calculated as the difference between the maximal RPT and the minimal RPT among the 3 layers.

**Statistical Analysis**

All measurements are presented as mean±SD. The one-way ANOVA was used to test differences in means between the each stage for statistical significance, with values p<0.05 considered indicative of statistical significance.
Fig 2. Time series of activation time (AT) maps and activation-recovery interval (ARI) maps estimated by 64-lead ventricle surface mapping (64 Map). The color scales are shown to the right of the figure. The numerical values at the bottom of the AT maps and ARI maps are the maximum and minimum values among the 64 leads. Dispersion of activation recovery interval (ARID) was calculated as the difference obtained by subtracting the minimum ARI value from the maximum ARI value, and it is shown in parentheses beneath the ARI values. CPR, cardiopulmonary resuscitation; NIF, nifekalant hydrochloride.

Fig 3. Results of 64-lead ventricle surface mapping (64 Map) in 8 dogs. (A) mean conduction velocity (Mean CV); (B) recovery time (Mean RT); (C) activation-recovery interval (Mean ARI); (D) min-ARI, minimum ARI; max-ARI, maximum ARI; ARID, dispersion of ARI. CPR, cardiopulmonary resuscitation; NIF, nifekalant hydrochloride.
Results

While repeatedly inducing VT/VF and performing electrical defibrillation after coronary artery ligation, arterial blood was collected each time the heartbeat resumed after defibrillation and was evaluated for acidosis. The pH of arterial blood 30 min after ligation was significantly lower than the control value before ligation, and the lactic acid values increased significantly and indicated metabolic acidosis (Table 1). The mean pH value 60 min after ligation was less than 7.2, indicating severe acidosis. NIF was administered 45±12 min after coronary artery ligation, and at that time the arterial pH was below the normal value, 7.26±0.03, and the lactic acid value was above normal, 52.1±24.3 mg/dl.

Effect of NIF on RR Interval and QT Interval Evaluated by Y-Lead ECG (Table 2)
There were no significant differences between the RR intervals or QT intervals at any of the times measured.

CT and ARID at the Epicardial Surface Evaluated by 64 Map
Representative data for AT and ARI of the 64 Map are shown in Fig 2. AT on the epicardial surface stabilized at a mean value of 5±1.1 ms at 1 h after insertion of the plunge electrode, but it was prolonged to 10±6.9 ms by ligation. However, little additional change was observed as a result of CPR or NIF (Fig 2 Upper panels). The mean ARI value 1 h after the plunge insertion was 223±2.7 ms, and it was slightly prolonged by ligation. However, the mean value had returned to 221±50.7 ms at 10 min after ligation, and after CPR it was prolonged again. After NIF administration, the mean value was further prolonged (Fig 2 Lower panels). ARID was 23 ms at 1 h after plunge insertion, but it increased to a maximum of 72 ms after ligation. It became worse after CPR; however, as a result of NIF administration ARID decreased significantly.

Total results of the 64 Map in the 8 dogs are summarized in Fig 3. CV was significantly delayed (34–46%) after ligation progression, but was not additionally delayed by CPR or NIF administration (Fig 3A). Mean RT, on the other hand, was prolonged by 8% immediately after ligation, 14% after CPR, and 25% after NIF administration (Fig 3B). There was no significant change in mean ARI as a result of ligation, but it was prolonged by 12% after CPR and 20% after NIF (Fig 3C). ARID was significantly increased from ligation to CPR, but it decreased markedly after NIF administration (Fig 3D, Right panel). The determining factor in the increases in ARID after ligation and after CPR was the significant prolongation of max-ARI (Fig 3D, Middle panel). By contrast, min-ARI decreased slightly as a result of ligation and CPR, but it was significantly prolonged by NIF (Fig 3D, Left panel), and ARID significantly improved as a result.
Dispersion of the RPT in the Epicardial Layer, Middle Layer, and Endocardial Layer of the LV Evaluated by Plunge Electrode

Representative waveforms of the 64 Map and plunge electrode in the LV are shown in Fig 4. Tpeak–Tend calculated by 64 Map were increased after ligation and after CPR, but marked improvement was observed after NIF administration (Fig 4, Top). Similar tendencies were seen in the changes of TDR when the plunge electrode was used (Fig 4, Bottom). At 1 h after plunge insertion RPT in the 3 layers was 276–284 ms, and the difference between max-RPT with min-RPT (plunge-TDR) was 8 ms. After ligation RPT was decreased in the subepicardium alone. After CPR marked prolongation of the RPT was observed in the subendocardium and subepicardium, but because it did not cause prolongation in the middle layer, Plunge-TDR was increased instead. After NIF, however, marked prolongation of RPT was observed in the middle layer alone, and Plunge-TDR was decreased.

The plunge potentials in 4 dogs are summarized in Fig 5. Because 10 min after ligation RPT significantly decreased

in the subepicardium alone, Plunge-TDR increased by 36%. After CPR, RPT was significantly prolonged in the subendocardium and subepicardium, Plunge-TDR increased by 90%. After NIF administration, however, significant prolongation of RFP was seen in the middle layer alone, and Plunge-TDR was improved.

The results for Tpeak and Tend calculated from 64 Map are shown in Fig 6. No significant changes in Tpeak and Tend were observed as a result of ligation. However, after CPR there was significant prolongation of Tend, but no significant change in Tpeak. NIF administration caused significant prolongation of both Tpeak and Tend, but it was greater on Tpeak. A review of these results in comparison with the Plunge-RPT values obtained with the plunge electrode (Fig 5) revealed that marked prolongation of Tend in 64 Map after CPR was determined in the subepicardium, while Tpeak was determined in the middle layer. Tend after NIF administration was determined in the subepicardium, and the marked prolongation of Tpeak was determined by the RPT prolongation in the middle layer.
Discussion

The major findings of this study are as follows. Induction of VT/VF and CPR were repeated after ligation of the proximal branch of the LAD, and NIF was administered 45 min later during the resulting acidosis. As a result, the LV surface and transmural RPT became homogeneous. Under the same conditions, however, NIF did not significantly prolong the QTc interval on the body surface Y-lead ECG. These findings suggest that the anti-arrhythmic action of NIF during CPR consists of suppressing reentry by organization of the RPT 3-dimensionally in the LV myocardium, rather than by the refractory-period-prolonging effect reported in the past. The homogenization of ARI at the ventricular surface is an effect attributable to administration of NIF increasing the min-ARI but having no significant effect on the max-ARI, and the uniform LV transmural RPT is an effect attributable to prolongation in the middle layer, where RPT is shortened the most, without affecting the RPT of the endocardial or epicardial layer where it was already increased by CPR. In regard to the Map-TDR calculated from Tpeak and Tend, it appears that transmural RPT became homogeneous because only Tpeak was prolonged by NIF administration.

Based on these findings, investigation of the degree of myocardial ischemia revealed a large dispersion transmurally, as well as at the heart surface, and that even 45 min after the onset of the ischemia RPT was prolonged by the endocardial layer and epicardial layer having more severe ischemia than the middle layer. NIF is thought to principally prolong RPT in the middle layer and to make RPT among the 3 layers homogeneous during CPR. Thus, the reason the QTc interval in the Y-lead recorded at the body surface was not prolonged by NIF may have been because the end of QT reflected the zone where the RPT of the ventricular muscle in which severe ischemia had developed was longest, and because even NIF administration did not prolong it any further in the zone where it was already long, it was not depicted as QTc prolongation. Another possible reason is that there was only 1-lead on the body surface ECG.

Significance of NIF in CPR

NIF is the first exclusively class III intravenous drug in Japan and is an excellent agent that shows promise of having a defibrillating effect on lidocaine-resistant refractory ventricular arrhythmias. The efficacy of NIF has been reported many times since being placed on the market in 1999.12-14 Because of the special pharmacological actions of NIF, primarily on IKr, its efficacy declines as stimulation frequency increases; in other words, it exhibits so-called reverse frequency-dependent blocking, and as a result there is a risk of causing severe QTc prolongation and torsade de pointes (TdP) during bradycardia.1 In other words, it is used carefully, with the possibility that the QT prolongation effect could become a serious side-effect. However, we observed significant resolution of VT/VF suppression by NIF, even though QT was not prolonged and because we could not explain the mechanism of VT/VF suppression by NIF, we thought another anti-arrhythmic effect might be hidden. This study is the first to examine whether NIF improves the TDR of the LV in a living animal, and provides new points of view that suggest an anti-arrhythmic effect of NIF apart from QT prolongation.

In our CPA model, significant QT prolongation was observed from at the 1.2 mg/kg dose. Metabolic acidosis was caused by an inhibition of the glycolytic system, increase in anaerobic pathways, decreased coronary artery blood flow, and electrolyte abnormalities (intracellular K+, Mg2+, Ca2+, etc), and thus it is unlikely that the drug will be used under such as complicated conditions. With regard to the pharmacological action of class III agents under acidic conditions, there is little information about NIF's pharmacokinetics, but AMD has been reported to remain unchanged. However it is known that intracellular Na+ increases as a result of delayed drug dissociation when lidocaine is used. Because NIF does not have negative chronotropic or inotropic effects like lidocaine,1,17 it can be said to be suitable for CPR. It is also noteworthy that NIF is an intravenous drug capable of reducing the electrical defibrillation threshold.18

Effect of Class III Anti-Arrhythmia Agents on TDR in the LV Myocardium

Information in the literature is sparse regarding prolongation of the APD after CPR, and the mechanism remains to be elucidated as to why only the mid layer fails to show APD prolongation. The coronary flow at the time of ischemia shows a greater decrease in the epicardium than the endocardium, and any change in the refractory period is greater in the epicardium than the endocardium. Because Ito and IKATP are predominantly distributed in the epicardium, RPT is easily affected by ischemia. Our study showed a shortening of the epicardial APD at the early stage of coronary ligation, whereas the APD was significantly prolonged as the hypoxic conditions progressed. These findings suggest that it is difficult to affect the mid layer coronary flow and APD by ischemia, and that a particular cell type existing only in the mid layer may provide some defense against environmental stress. If we assume that some sort of protective effect exists in the mid layer against severe hypoxia-related cell damage, then the APD prolongation by NIF is produced in the mid layer because ion channel function is maintained. Antzelevitch et al have reported on the effect of class III drugs on the TDR in wedge models in normal dogs.10,19,20 Sicouri et al stated that chronic oral administration of AMD in dogs reduced the TDR by causing APD prolongation in the epicardium and endocardium of the LV, and APD shortening in M cells.19 On the other hand, when they administered Ikr blockers, such as E-4031, and d-sotalol, there was a relative increase in the TDR as a result of preferential APD prolongation in the M cells.19,20

Although the APD fluctuation seen in the present study was incompatible with the general idea suggested by Antzelevitch et al, the improvement of the TDR by class III drugs is a very important electrical mechanism for developing a defibrillatory effect. As for the lack of concordance between our results and Antzelevitch's theory, there are 3 possible explanations: (1) Antzelevitch used a wedge preparation model whereas we used an in vivo canine model, in which factors such as endocrine or autonomic nerve regulation can exert an influence on the results; (2) we used a severe ischemic model complicated with hypoxia and acidosis; and (3) we used NIF, which is a pure IKr blocker acting as a K+ channel blocker, and not sotalol or AMD. These differences in the methods might have had some effect on the results and it poses new problems that need to be elucidated in the future.

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Combined Action of NIF and Catecholamines During CPR

It has been reported that the combination of an IKr blocker (E-4031) and β-receptor agonist (isoproterenol) produces an APD alternation phenomenon (shortening and prolongation) in rabbit papillary muscle tissue section, and that TdP tended to develop as a result of the instability of repolarization. Moreover, another report using living dogs stated that the incidence of adrenaline-induced TdP was increased by the combined effect of MS-551 and an β, β-receptor agonist (adrenaline). Even if a β-receptor agonist is administered alone without NIF, once myocardial ischemia develops, -adrenaline sensitivity becomes locally inhomogeneity at the epicardial border zone and causes alternation of the RPT with each heartbeat in addition to spatial dispersion. However, in the present study we did not see alternation of the RPT when NIF was administered after epinephrine (data not shown) and we consider that one of the reasons for this result is the background conditions of the CPA. Selective binding ability of β-2 receptors decreases with the severity of the acidosis and expression of β-receptor function is relatively low because it is a state in which endogenous adrenaline secretion is greatly inhibited, even if exogenous epinephrine is added during CPR. Moreover, all ion channels, pumps, and transporters in the myocardium temporarily stop during CPA, and even if K currents are partially reversed as a result of restoration of the heartbeat, it is difficult to say that they exhibit complete recovery of function. Thus, when catecholamines or IKr blockers are administered, it is necessary to consider the possibility that the absolute number of channels is less or that the binding ability of the channels themselves is reduced.

Conclusion

Administration of NIF during CPR reduced the TDR by prolonging the RPT of the middle layer of the LV myocardium. These results suggest that the QT-prolonging effect that NIF possesses may not be reflected in the body surface ECG because the RPT in the endocardium and epicardium after CPR is already prolonged before NIF administration.

Acknowledgments

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References


